Interim Report

USE OF MYCORRHIZAL INOCULATION FOR PLANT ESTABLISHMENT
ON HIGHWAY SITES

TO: H. L. Michael, Director
Joint Highway Research Project

August 13, 1981
Revised February 2, 1982

Project: C-36-48H

FROM: David F. Hamilton
Department of Horticulture

File: 9-5-8

Attached is the first Interim Report on the HPR Part II Study titled "Techniques to Increase Survival of New Highway Plantings". This report concerns that part of the project concerned with the critical effects associated with the use of mycorrhizae during plant establishment. The title of the report is "Use of Mycorrhizal Inoculation for Plant Establishment on Highway Sites". Messrs. S. D. Verkade and D. F. Hamilton are the authors of the report and the investigators on this part of the study.

Important findings are that mycorrhizae increase the growth and survival of plants by improving nutrient and moisture uptake. Development of inoculation programs could result in substantial savings through greater survival of landscape plants.

The report is presented for review and acceptance as partial fulfillment of the objectives of the noted HPR Study. Additional reports on the other phases of the project are in preparation.

Respectfully submitted,

David F Hamilton/HLM.

David F. Hamilton
Department of Horticulture

DFH:ms

cc: A. G. Altschaeffl
W. L. Dolch
B. L. Elkin
R. L. Eskew
G. D. Gibson
W. H. Goetz
M. J. Gutzwiller
G. K. Hallock
D. E. Hancher
J. F. McLaughlin
R. D. Miles
P. L. Owens
G. T. Satterly
C. F. Scholer
K. C. Sinha
C. A. Venable
L. E. Wood
E. J. Yoder
S. R. Yoder

Interim Report

USE OF MYCORRHIZAL INOCULATION FOR PLANT ESTABLISHMENT ON HIGHWAY SITES

by

Stephen D. Verkade

David F. Hamilton

Department of Horticulture
Purdue University

Joint Highway Research Project
Project No.: C-36-48H
File No.: 9-5-8

Prepared as Part of an Investigation

Conducted by

Joint Highway Research Project
Engineering Experiment Station
Purdue University

in cooperation with the

Indiana State Highway Commission

and the

U.S. Department of Transportation
Federal Highway Administration

Purdue University
West Lafayette, Indiana
August 13, 1981
Revised February 2, 1982

TECHNICAL REPORT STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
FHWA/IN/JHRP-81/13		

4. Title and Subtitle USE OF MYCORRHIZAL INOCULATION FOR PLANT ESTABLISHMENT ON HIGHWAY SITES	5. Report Date August 13, 1981 February 2, 1982 (Revised) 6. Performing Organization Code
7. Author(s) Stephen D. Verkade David F. Hamilton	8. Performing Organization Report No. JHRP-81-13
9. Performing Organization Name and Address Joint Highway Research Project Civil Engineering Building Purdue University West Lafayette, Indiana 47907	10. Work Unit No. 11. Contract or Grant No. HPR-1(19) Part II
12. Sponsoring Agency Name and Address Indiana Department of Highways State Office Building 100 North Senate Avenue Indianapolis, Indiana 46204	13. Type of Report and Period Covered Interim Report Mycorrhizal Phase 14. Sponsoring Agency Code

15. Supplementary Notes

Prepared in cooperation with the U.S. Department of Transportation, Federal Highway Administration. Part of HPR-1(19) Part II Study titled "Techniques to Increase Survival of New Highway Plantings".

16. Abstract

Three highway soils were tested for presence of spores from mycorrhizal fungi. A newly disturbed soil had virtually no mycorrhizal spores present, while a soil successfully revegetated with herbaceous species and one revegetated with herbaceous and woody species had very high numbers of mycorrhizal spores.

Compatibility of plants, commonly used in highway plantings, with mycorrhizal fungi was also examined. Growth of *Liriodendron tulipifera* was promoted by association with *Glomus fasciculatus*, and growth of *Ligustrum obtusifolia Regelianum* was promoted by *Glomus mosseae*. However, growth of *Acer platanoides* and *Lolium perenne* were not improved by mycorrhizal association with *G. fasciculatus*. Growth of *Forsythia intermedia* was not improved by association with *G. mosseae*. Other fungi symbionts may promote the growth of these plants.

Inoculum for plants used in highway plantings should include many species of fungi, to compensate for any host specificity. Mycorrhizae increase the growth and survival of plants by improving nutrient and moisture uptake. Development of inoculation programs for plants to be used in highway plantings could result in substantial savings of money spent for establishment of landscape plants in the revegetation of disturbed sites.

17. Key Words	18. Distribution Statement
Roadside Plantings, Landscape Plantings, Mycorrhizae, Spores	No restrictions. This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	12	

Form DOT F 1700.7 (8-69)

HIGHLIGHT SUMMARY

Following construction of a new highway, the soil is unavoidably disturbed, providing an unfavorable environment for the reestablishment of plants on these sites. These locations are generally characterized by high temperature, low moisture, poor fertility, and lack of beneficial mycorrhizal fungi. Development of mycorrhizal roots could improve survival and growth of plants in new highway plantings.

Newly disturbed soils had no mycorrhizal spores present, while successfully revegetated highway soils had large populations of mycorrhizal fungi. Mycorrhizae promote growth of plants by improving moisture and nutrient uptake, resulting in increased growth and survival.

A plant species may not be compatible with all species of mycorrhizal fungi. Growth of *Liriodendron tulipifera* (tulip poplar)was improved by association with *Glomus fasciculatus*, and growth of *Ligustrum obtusifolium Regelianum* (Regel privet) was enhanced by *Glomus mosseae*. However, growth of *Acer platanoides* (Norway maple) and *Lolium perenne* (perennial ryegrass) was not improved by *G. fasciculatus*, and growth of *Forsythia intermedia* (Forsythia) was not greatly improved by association with *G. mosseae*. Other species of fungi may improve growth of these plants.

Mycorrhizal inoculum for highway sites should include many species of fungi to insure compatibility with the range of plants used in highway plantings. Inoculation of plants used in highway plantings could improve growth and survival of these plants. A successful inoculation program could result in substantial savings of plant material and fertilizers used to revegetate highway sites.

Table of Contents

List of Tables

INTRODUCTION

After the construction of a new highway, the soils remaining are unavoidably disturbed and provide harsh environments for the reestablishment of vegetation on these sites. Unfavorable aspects of the newly established highway locations often include high temperature, low or excess moisture, poor fertility, and lack of beneficial soil microorganisms. These soils are usually subsoils or exposed hardpans.

The high temperatures are a severe detriment to the establishment and survival of young landscape plants. Damaging temperatures as high as 100-115°F are common on slopes adjacent to highways. The growth of many herbaceous and woody plants is limited by temperatures above 85°F. Since it is not feasible to provide shade on the large scale required on highways, plants adapted to survival under these conditions are selected with limited success.

The problem of high temperatures at highway soil sites is compounded by the low moisture content of many soils. Several factors contribute to the poor water relations of these sites. First, the physical disruption of the soil can permenantly alter the soil structure, resulting in less effective use of the water table to supply plants with moisture. Secondly, since there initially is no vegetative cover, loss of moisture by evaporation at the soil surface is maximized. Finally, since there is no shading, temperatures are abnormally high, also promoting evaporation. However, hot and dry conditions are not the only problems to overcome when reestablishing vegetation at a highway site.

Limited availability of plant nutrients (mainly nitrogen, phosphorus, and potassium) is also a serious and costly problem. Many times, subsoils with extremely low nutrient levels are exposed at a new site which must be revegetated. The problem of low nutrient content is complicated by the

leaching effect of rains on slopes. To deal with the problem of low fertility, expensive fertilizers are applied.

As energy costs increase and the supply of raw materials becomes limited, fertilizer is becoming less available and more expensive. This trend makes it imperative that waste be eliminated and that all fertilizers applied be utilized efficiently.

Related to all three of these problems is a lack of beneficial soil microbes in harsh highway soils. The soils under consideration are often disturbed subsoils with microbe populations generally less than normal. Ultimate establishment and growth of the plant may depend on higher than normal populations of the beneficial soil microorganisms. Perhaps the most potentially useful of the beneficial soil microbes are symbiotic mycorrhizal fungi.

Certain soil fungi combine in symbiosis with plant roots to form a relationship termed mycorrhizae (pronounced: my-kor-īzee), in which both symbionts benefit. There are two types of mycorrhizae, which differ in the way in which the fungi attach to the root. The fungi of the ectomycorrhizal group form a hyphal mantle around the exterior of the root, resulting in a fuzzy appearance. The fungi also enter the root by passing in between the individual cells, but do not enter the individual cells.

The second type is the endomycorrhizal group, which differs from the ectomycorrhizal group in two repsects. First, endomycorrhizae have no fungal mantles on the exterior of the root tip, although the hyphae do extend outward into the soil. Secondly, the hyphae inside the root do penetrate the individual cells and cause swelling. Thus, endomycorrhizal formation is only detectable when viewed under a microscope.

Although both types are symbiotic, endomycorrhizae appear to be the most

critical for growth of landscape plants. Endomycorrhizal fungi benefit from the relationship by obtaining carbohydrates from the plant, while contributing to plant health by increasing nutrient and moisture uptake. The increases in uptake of moisture and nutrients are due to the absorptive surfaces added by the fungal hyphae, and perhaps to more efficient uptake by the hyphae. Plants with mycorrhizal roots have a distinct advantage over nonmycorrhizal plants for establishment on harsh sites, because they are better adapted to absorb the limited supply of moisture and nutrients. These aspects can be critical factors in the establishment of landscape plants on adverse highway sites, by enabling the plants to tolerate a wider range of environmental conditions.

To make maximum use of mycorrhizal inoculation of landscape plants for highway sites, it is imperative to determine the role that mycorrhizal fungi play in the successful establishment of these plants on harsh sites. In addition, since not all mycorrhizal fungi are compatible with all plant species, it is also important to be aware of the plant-fungi compatibility.

One purpose of this study is to determine the number of spores present before and after revegetation, as a measure of the importance of mycorrhizae in the reestablishment of plants on these sites. Another purpose of this study is to examine the compatibiltiy of certain plants used in highway plantings with selected mycorrhizal fungi.

MATERIALS AND METHODS

Experiment 1. The number of spores were estimated in a newly disturbed soil, a highway soil revegetated with herbaceous plants, and a highway soil revegetated with herbaceous and woody plants. On the newly disturbed site, no vegetation was present. The second site had been successfully revegetated for

approximately eight years with herbaceous plants including *Lolium perenne*, *Poa partensis*, and *Coronilla varia*. The third site had also been successfully revegetated for approximately eight years, but in addition to the herbaceous species, it also had the woody plants *Viburnum dentatum*, *Lonicera morrowii*, and *Fraxinus pennsylvanica*. All three sites were located in West Lafayette, Indiana.

Ten soil samples were taken randomly at each location from the top 6 cm of soil. For each site, the soil samples were mixed to form a composite sample, before being subsampled for the spore counts. From each composite soil sample, 8 one-gram subsamples were examined for content of spores from mycorrhizal fungi. Spore quantification was done using the sucrose centrification method of Jenkins (1964). Analysis of variance was conducted using the Newman-Keuls test of statistical significance.

Experiment 2. Five plant species were tested to determine the compatibility of plant and fungal species. They were *Lolium perenne* tested with *Glomus fasciculatus*, *Forsythia intermedia* with *Glomus mosseae*, *Acer platanoides* with *G. fasciculatus*, *Liriodendron tulipifera* with *G. fasciculatus*, and *Ligustrum obtusifolium Regelianum* with *G. mosseae*.

Seeds of *A. platanoides* and *L. tulipifera* were sown in a medium of 1 perlite:1 vermiculite by volume and placed in a growth chamber (26° C $\pm$ 2° C with a 13-hour photoperiod) for four weeks. Terminal cuttings of *F. intermedia* were taken 20-cm long, with unhardened growth removed, and rooted in a medium of 1 sand:1 perlite by volume under intermittent mist (15 seconds per 10 minutes). Indolebutyric acid (0.1%) in talc was used to enhance rooting.

All seedlings and cuttings were transplanted into 3.28 liter pots (one gallon trade designation) containing steam pasteurized medium (1 perlite:1 peat moss:1 soil, by volume). The unfertilized medium contained 0.09 g N/l, 0.01 g

P/l, 0.04 g K/l and had a pH of 6.5. All plants were fertilized with 2 g N/l of "Osmocote" 19-6-12 slow release fertilizer. Seeds of L. perenne were sown directly into the 3.28 liter pots, at a rate of 185 gms/15 m^2, and covered with 0.5 cm of soil. Pots were watered with tap water as needed.

After 21 weeks, the woody plants were measured to determine height increase and their roots stained to examine mycorrhizal development. The L. perenne plants were harvested after five weeks and shoots were weighed. The amount of myorrhizal infection was evaluated by visual estimation using root staining and microscopy (Phillips and Hayman, 1970).

Cuttings of L. obtusifolium Regelianum were rooted either in a medium of 1 vermiculite:1 perlite, or in this medium amended with mycorrhizal inoculum (3 medium:1 inoculum, with 40,800 spores added to a flat 35 X 42.5 X 12.5 cm). The cuttings were placed under intermittent mist in a greenhouse with approximately 25% shade. Cuttings were harvested after 9-10 weeks. Analyses included fresh weight of roots to examine promotion of growth by mycorrhizae, and root stains to exmine mycorrhizal development. Analysis of variance was conducted with the Newman-Keuls test of significance.

RESULTS AND DISCUSISON

Mycorrhizae do play a role in the revegetation of harsh sites. The newly disturbed soil had virtually no spores present. However, the soils that were successfully revegetated had much higher numbers of spores from mycorrhizal fungi (Table 1). There was no difference between the number of spores found in the soil revegetated with herbaceous species, and those found in the soil revegetated with herbaceous and woody species. This indicates that initially

there are no mycorrhizal spores present in highly disturbed soils, but mycorrhizal development is important for the reestablishment of landscape plants on harsh highway sites. Both woody and herbaceous plant species appear to rely on the formation of mycorrhizae.

Mycorrhizal inoculation did not promote growth of all the combinations of plant and fungal species. Although not all of the plant species tested benefited from association with the fungal species used, there was no case in which the plant was harmed. Forsythia, maple, and perennial ryegrass had minimum mycorrhizal development (less that 2% of the roots affected), and had no significant growth promotions due to the presence of the mycorrhizal fungi tested with those species (Table 2). Although these plants did not benefit from association with the fungal species tested, they may benefit from mycorrhizal association with other fungal species.

Growth of tulip poplar was significantly promoted by mycorrhizal development. The roots of inoculated plants were highly mycorrhizal (more than 50% of the roots affected), with the height of inoculated plants greater than those of nonmycorrhizal plants. Growth of regel privet was also enhanced by mycorrhizal development. The roots of cuttings of regel privet were highly mycorrhizal, and the fresh weights of roots of cuttings rooted in the medium amended with mycorrhizal inoculum were significantly greater than those of cuttings rooted in media not amended with mycorrhizal inoculum.

Formation of mycorrhizal roots can be critical to the survival of a landscape in the unfavorable environment of the highway site. Conditions at highway sites often include high temperatures, low moisture availability, and very low plant nutrient supplies. These problems are complicated by the lack of reproductive spores of mycorrhizal fungi in these soils. Because of the ability of mycorrhizae to increase plant growth by enhancing moisture and

nutrient uptake, inoculation with mycorrhizal fungi can be an important management technique for highway revegetation. Mycorrhizal inoculum is available coomercially in limited amounts from Abbott Laboratories, Long Grove, Illinois.

The formation of mycorrhizal roots is very common for many healthy plant species in nature. Some plant species commonly used on Indiana highway sites which benefit from mycorrhizal association include members of the genera:

1. <u>Acer</u>
2. <u>Alnus</u>
3. <u>Eleagnus</u>
4. <u>Festuca</u>
5. <u>Fraxinus</u>
6. <u>Liquidambar</u>
7. <u>Lolium</u>
8. <u>Myrica</u>
9. <u>Nyssa</u>
10. <u>Pinus</u>*
11. <u>Platanus</u>
12. <u>Populus</u>*
13. <u>Quercus</u>*
14. <u>Salix</u>*
15. <u>Vaccinum</u>
16. <u>Viburnum</u>

*These form ectomycorrhizal relationships, other form endomycorrhizal.

Since the availability of a large and dynamic population of mycorrhizal fungi plays a role in the establishment of plants in highway revegetation, early development of mycorrhizal roots could increase the survival of plants on highway sites. Compatibility of the plant and fungi is an important criteria of mycorrhizal symbiosis, and inoculum sources should contain several species of spores to accomodate the variety of plants commonly used in highway plantings.

If plants with mycorrhizal roots are selected for highway plantings, inoculation may not be necesssary. However, current plant production systems often include the use of media without soil or mycorrhizal fungi. If the plants used do not have mycorrhizal roots, inoculation may be used to increase growth and survival.

Inoculation of plants can be implemented in either of two phases. If the plants are inoculated during the production phase, the earliest development of mycorrhizal roots is achieved. However, if the plants used to revegetate highway sites are nonmycorrhizal, inoculation can be included when the planting or seeding is done.

In summary, highway locations are highly disturbed and provide very unfavorable conditions for the establishment of plants during revegetation. Mycorrhizal inoculation may be useful for improving the growth and survival of landscape plants on highway sites, reducing the requirement for fertilizers. The importance of mycorrhizal inoculation would be to reduce the expense of revegetating highway sites by reducing the loss of plant material and the requirement for fertilizer additions.

With increasing costs of plant material, fertilizers, energy, and labor, more efficient use of resources will become increasingly important in the future. Successful development of mycorrhizal inoculation programs for plants used in highway plantings could result in large savings of limited financial resources spent on plant material and fertilizers.

Conclusions:

The following conclusions can be drawn from this study:

1) Newly disturbed highway soils studied had virtually no nycorrhizal spores present, while successful revegetation of highway sites corresponds to the reestablishment of high spore numbers in the soil. Therefore, it is likely that mycorrhizae do play a role in the revegetation of harshly disturbed sites. Development of mycorrhizae has been found to enable the plant to take up moisture more readily and to utilize nutrients more efficiently.

2) This study indicates that the growth all species of plants are not promoted by the same mycorrhizae fungi. Therefore, mycorrhizal inoculation used for highway sites should include several fungal species, to accomodate the various plants important in highway revegetation.

Table 1. Numbers of mycorrhizal spores present in a newly disturbed soil, a highway soil revegetated with herbaceous plants, and one revegetated with woody and herbaceous plants.[z]

Soil Condition	Number of Mycorrhizal Spores per gram of soil
Newly Disturbed	0.1[a]
Revegetated with Herbaceous plants	24.3[b]
Revegetated with Herbaceous and Woody Plants	32.6[b]

[z] Separation of means by the Newman-Keuls test of significance, 5% level. Mean of eight values used.

Table 2. Growth and symbiotic compatibility of selected landscape plants inoculated with mycorrhizal fungi, under greenhouse conditions.[z]

Plant Species	Fungal Species	Growth Measurement	Inoculated	Noninoculated	Compatibility
Acer platanoides[y]	*Glomus fasciculatus*	Height Increase (cm)	55.4	43.4	-
Forsythia intermedia[y]	*G. mosseae*	Height Increase (cm)	71.9	65.1	-
Ligustrum obtusifolium Regelianum[x]	*G. mosseae*	Fresh Wt. of Roots (gms)	0.487	0.215	+
Liriodendron tulipifera[y]	*G. fasciculatus*	Height Increase (cm)	36.9	3.4	+
Lolium perenne[y]	*G. fasciculatus*	Dry Wt. of Shoots (gms)	2.98	3.08	-

z - Seperation of means by the Newman-Keuls test of significance, 5% level.

y - Mean of 13 values, plants grown at 2 gm N/1 fertility level.

x - Mean of 8 values, cuttings under intermittent mist.

1. Hayman, D. S. 1978. Endomycorrhizae, In: Interactions Between Non-pathogenic Soil Microorganisms and Plants. Y. R. Dommergues and S. V. Krupa (Eds.) Elsevier Scientific Publishing Co., New York. pp. 401-441.

2. Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. Plant Disease Reporter. 48(9):692.

3. Phillips, J. M. and D. S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbusculor mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55(1):158-161.

COVER DESIGN BY ALDO GIORGINI